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Full Length Research Paper

Is the Dutch domesticated strain of *Clarias gariepinus* (Burchell, 1822) a hybrid?

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In a study of genetic characteristics of exotic *Clarias gariepinus* we obtained the animals from five hatcheries in Nigeria and sequenced 399 base pair of the cyt b gene. The results show that the exotic *C. gariepinus* clustered in two lineages: *Clarias anguillaris* (West Africa) and *C. gariepinus* (East Africa). First, this strain could be a hybrid between the two species since the original populations used for selection were from different sources including West Africa where both occur sympatrically. Alternatively, it has been contaminated via unwholesome practices.

Keywords: *Clarias gariepinus*, Holland Clarias, contaminated hatchery stock and hybrid.

INTRODUCTION

The Clariid catfishes, order Siluriformes, are distributed in Africa, Asia Minor and South-east Asia and the Indian subcontinent (Teugels and Adriaens, 2003). Two genera of this family, *Clarias* and *Heterobranchus*, with the Cichlids are the most used in African aquaculture (Agnese et al., 1995).

The *Clarias*(*Clarias*) (Teugels, 1982, 1986) made up of only two valid species, *Clarias gariepinus* and *Clarias anguillaris* which cannot be differentiated morphologically except by counting the number of gill rakers on the dead fish dominates the Nigerian aquaculture scene. While *C. gariepinus* occurs throughout Africa, *C. anguillaris* is geographically restricted to West Africa where they occur sympatrically. *C. gariepinus* was introduced into The Netherlands in the 1970's from different sources: Cote d'Ivoire, Central Africa, Cameroon and Israel (Huisman and Richter, 1987; Richter et al., 1987; Welcomme, 1988; Holcik, 1991). They were selected for fast growth and conformation (Cambray and Van der Waal, 2006 and references therein; Huisman and Richter, 1987; Richter et al., 1987) and these are being reintroduced to different African countries. The government of Nigeria through the Nigeria Institute of Freshwater Fisheries Research in collaboration with the World Bank sponsored National Agricultural Development project imported this Dutch domesticated strain (Dada and Wonah, 2003) over a decade ago. Since the introduction, no study has been carried out to determine its genetic characteristics. Mitochondrial DNA sequences have been employed in the characterization and identificaion of organisms, determination of species origin and tracking of released animals (Ferris et al., 1982; Partis and Well, 1996; Wolf et al., 2000; Gao et al., 2001; Fujii, 2002). The objective of this study was therefore to characterize and identify the species status of the exotic *C. gariepinus* using mitochondrial DNA.

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MATERIALS AND METHODS

Fish samples and DNA extraction

Exotic *C. gariepinus*, that is, the Dutch strain were obtained from five hatcheries in Nigeria. Samples of *C. albopunctatus* Nichols and La Monte, 1953, and *C. anguillaris* Linn., 1758 were obtained from fishermen at the Anambra River basin and were identified by Dr. J. N. Aguigwo of the Fisheries Department, Nnamdi Azikiwe University, Awka, Nigeria. Table 1 shows the sample size for each population. We considered all samples of exotic *C. gariepinus* to be genetically pure since there was no recorded information of contamination. Muscle tissues of specimen were obtained and fixed in 95% alcohol. To extract DNA, muscle tissue was digested with proteinase K followed by extraction with phenol-chloroform-isoamyl alcohol. The DNA was precipitated in absolute alcohol and the pell-

Table 1. Summary of specimen, sources and abbreviations.

S/N	Specimen	Abbrev	Source	Size (n)
1	*Heterobranchus longifilis*	HET	Delta State	2
2	*Clarias albopunctatus*	CALB	Anambra River Basin	3
3	Holland Clarias(*C. gariepinus*)	LAG	Cisco	3
4	Holland Clarias(*C. gariepinus*)	DT	Songhai Delta	2
5	Holland Clarias(*C. gariepinus*)	IBA	Zartech	2
6	Holland Clarias(*C. gariepinus*)	LA	Lagos	2
7	Holland Clarias(*C. gariepinus*)	KA	Kainji	2
8	*Clarias anguillaris*	CLZ	Anambra River Basin	2
9	*Clarias anguillaris*	CAN	Kainji lake area	2
10	*Clarias anguillaris*	CSUB	Anambra River Basin	2
11	*C. anguillaris*	AF126824	Agnese and Teugels, 2001	
12	*C. gariepinus*	AF126823	Agnese and Teugels, 2001	
13	*C. buettikoferi*	AF126829	Agnese and Teugels, 2001	
14	*C. ebriensis*	AF126822	Agnese and Teugels, 2001	
15	*Gymnallabes typus*	GMN	Delta State	
16	Unidentified	UKN	Anambra River Basin	

ets washed with 70% ethanol. This was dried and resuspended in 100 µl of distilled water.

DNA amplification and sequencing

Part of double stranded Cytochrome b gene template of specimens suitable for sequencing were obtained by the Polymerase Chain Reaction, PCR, using the universally conserved mtDNA cyt b primers: L14734-GLU (5'- AAC CAC CGT TGT TAT TCA ACT-3') and H15149 cyt b (5'- CTC AGA ATG ACA TTT GTC CTCA-3') (Inoue et al., 2001; Kocher et al., 1989).

The PCR was performed in 50 µl volumes containing 1.25 units of Taq Polymerase (Tarkata, Dalian, China), 200 $nmolL^{-1}$ forward and reverse primers, 200 $\mu molL^{-1}$ each of dNTPs, 10 $mMolL^{-1}$ Tris (pH 8.3), 50 $mMolL^{-1}$ KCl, 1.5 $mMolL^{-1}$ $MgCl_2$. PCR cycling conditions were: 3 min initial denaturation at 94°C and 40 cycles of 45 s at 94°C for denaturation, 45 s at 50°C for annealing, 45 s at 72°C for extension, and a final extension of 10 min at 72°C. We included a control to monitor contamination.

The size of PCR product was verified by electrophoresis in 1% Agarose gel using DNA marker DL2000 (Tarka Co., Dalian, China). The amplified cytochrome b gene was isolated and purified using Gel Extraction Minikit (Watson Biotechnologies Inc., Shanghai). The purified PCR products were cycle-sequenced with PE Biosystems Big Dye Terminator cycle sequencing kit and run on ABI Prism 377 (Applied Biosystems, USA) automatic sequencer.

Sequence analysis.

The sequences were edited and aligned using DNASTAR software (DNASTAR Inc.). Genetic distances were generated using Kimura's two-parameter substitution model. Codons in the nucleotide sequence data were translated to their corresponding amino acids and nucleotide compositions were calculated. For the phylogenetic analysis, known sequences of *Clarias buettikoferi* (AF126829), *C. gariepinus* (AF126823), *C. ebriensis* (AF126822) and *C. anguillaris* (AF126824) were downloaded from the NBCI GenBank as references. All the analysis was carried out with the software MEGA 3.1 (Kumar et al., 2004).

RESULTS

The sequence data of 399-bp segments at the 5' end of cytochrome b gene excluding the 13 base pair partial segment of tRNA-GLU gene was determined for 5 populations of Exotic *C. gariepinus*. The nucleotide composition for the 399 base pair showed a deficit of G (< 15%) in favour of C and A over T. There was also a pronounced bias towards A and C (> 83%) at the third codon position of the genetic code. Base composition at the second codon composition was highly conserved for all specimens of exotic *C. gariepinus*.

In the 399 base sequences for the in-group (all exotic *clarias* only), 13 (3.3%) sites were polymorphic, 11 of these (84%) were phylogenetically informative. Table 2 shows the variable sites among the exotic *Clarias* cultured in Nigeria. The ratio of transition to transversion was 1:6. All the substitutions were at the third codon position and silent.

Figure 1 showed the phylogenetic tree recovered from the partial sequence of cytochrome b gene of exotic *clarias* from 5 farms, *Heterobranchus longifilis*, *Gymnallabe typus* and other *Clarias* species (Table 1) according to the neighbour-joining method. The tree of the exotic *clarias* revealed 2 distinct evolutionary lineages: West African and East African. 60% of the exotic *Clarias* clustered to the East African lineage which included *C. gariepinus* of Genbank sequence AF126823. The West African lineage to which 40% of the specimens clustered included all the species of *C. anguillaris* taken from the wild and Genbank sequence AF126824. The average genetic distance (d, Kimura 2-parameter) was 0.051 for the in-group, ranging from 0.0076 to 0.0970. The highest pair wise distance was 0.4384 recorded between all exotic *Clarias*, which paired to *C. anguillaris* (AF126823)

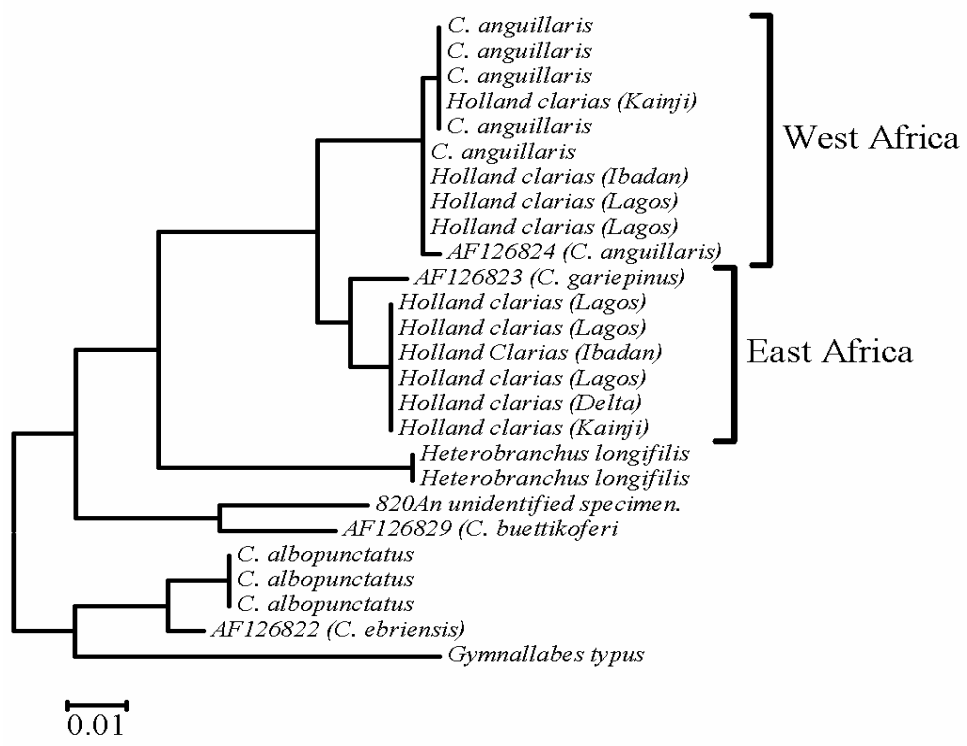

Figure 1. Phylogenetic relationship among *Clarias* species.

and *G. typus* (GMN) and followed closely by a distance of 0.4305 between the GMN and *C. anguillaris* (Table 3). Analysis of the West African lineage revealed 2 polymorphic sites and a mean pair wise genetic distance of 0.0059. One of the substitutions was transversional. There was no variation in the East African clade, which may suggest one or a very narrow source.

When *Clarias albopunctatus* was taken into account, the number of ploymorphic sites increased considerably to 46, 44 of the sites being parsimony informative. Between East African (*C. gariepinus*) and *C. albopunctatus* and the West African lineage and *C. albopunctatus* were 40 variable sites, respectively. All the sites were parsimony informative. The overall average genetic distance was 0.16. 32.5% (13) of the 40 variable sites between *C. albopunctatus* and *Clarias* of the West African lineage were transversions. We also recorded 35 transitions and 7 transversions between *H. longifilis* and *C. albopunctatus* with all the 42 polymorphic sites phylogenetically informative. There were 37 and 31 variable sites between *Heterobranhus* and West African and *Heterobranchus* and East African clades, respectively, with all the polymorphic sites informative.

DISCUSSION

The base composition of cytochrome b sequences agrees with description for many animals and fishes where a global deficiency of guanine has been observed (Agnese and Teugels, 2005; Brown, 1983, 1985; Cantatore et. al., 1994; Liu et al., 2005; Meyer, 1993). On the third codon position, Exotic *Clarias* showed an extreme global deficit of guanosine (0.8 – 2.3%) which was in agreement with results obtained in the Cichlid, *Hemichromis bimaculatus* and the American eel, *Anguilla rostrata* (Meyer, 1993), *Acipenser transmontanus* (Brown et al., 1979), and Japanese grenadier anchovy, *Colia ectenes* (Liu et al., 2005).

The exotic *C. gariepinus* is marketed in Nigeria under different names: Exotic *C. gariepinus*, Albino *Clarias*, Holland or Dutch *Clarias* and it constitutes one of the major strains of the large African catfish cultivated. *C. gariepinus* was thought to be the progenitor of exotic *C. gariepinus* (Dada and Wonah, 2003), which has undergone many years of selection and domestication (Cambray and Van Der Waal, 2006 and references therein; Huisman and Richter, 1987). One of the problems of aquaculture development in Nigeria is that native species have not been selected and domesticated enough to be acclima-ted and suitable for rearing conditions. Thus, response to management is limited. High growth rate (6%) in the catfish *Ictalurus punctatus* from generation to generation was attributed to long period of domestication and adaptation to rearing condition (Dunham et al., 2001).

Based on our hypothesis of genetic purity and lack of contamination of all samples of exotic *C. gariepinus*, evid-

Table 2. Cytochrome b variable sites and nucleotide changes found in exotic *Clarias gariepinus*

Specimen	96	102	135	141	171	211	231	270	297	303	309	330	333	342	348	384	399
LA	T	A	A	A	T	G	C	C	T	G	T	G	C	C	C	A	A
LA	T	A	A	A	T	G	C	C	T	G	T	G	C	C	C	A	A
IBA	T	A	A	A	T	G	C	C	T	G	T	G	C	C	C	A	A
LAG	T	A	A	A	T	G	C	C	T	G	T	G	C	C	C	A	A
DT	T	A	A	A	T	G	C	C	T	G	T	G	C	C	C	A	A
KA1	T	A	A	A	T	G	C	C	T	G	T	G	C	C	C	A	A
AF126823	T	A	C	A	C	T	C	T	T	C	T	A	C	C	C	A	A
CAN	C	T	C	T	C	T	T	T	C	T	C	A	C	T	T	A	A
AF126824	C	T	C	T	C	T	T	T	C	T	C	A	C	T	T	A	G
IBA	C	T	C	T	C	T	T	T	C	T	C	A	C	T	T	A	G
CAN	C	T	C	T	C	T	T	T	C	T	C	A	C	T	T	A	G
LAG	C	T	C	T	C	T	T	T	C	T	C	A	C	T	T	G	G
CLZ	C	T	C	T	C	T	T	T	C	T	C	A	A	T	T	A	G
KA1	C	T	C	T	C	T	T	T	C	T	C	A	A	T	T	A	G
CLZ	C	T	C	T	C	T	T	T	C	T	C	A	A	T	T	A	G
CSUB	C	T	C	T	C	T	T	T	C	T	C	A	A	T	T	A	G
CSUB	C	T	C	T	C	T	T	T	C	T	C	A	A	T	T	A	G

Table 3. Pair wise genetic distance for exotic Clarias gariepinus.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
HET																			
LA	0.2443																		
IBA	0.2443	0.0000																	
LAG	0.2443	0.0000	0.0000																
DT	0.2443	0.0000	0.0000	0.0000															
KA	0.2443	0.0000	0.0000	0.0000	0.0000														
AF126823	0.2245	0.0387	0.0387	0.0387	0.0387	0.0387													
AF126824	0.2925	0.0970	0.0970	0.0970	0.0970	0.0970	0.0980												
IBA	0.2800	0.0882	0.0882	0.0882	0.0882	0.0882	0.0891	0.0076											
CAN	0.2800	0.0882	0.0882	0.0882	0.0882	0.0882	0.0891	0.0076	0.0000										
LAG	0.2925	0.0970	0.0970	0.0970	0.0970	0.0970	0.0980	0.0153	0.0076	0.0076									
KA	0.2905	0.0966	0.0966	0.0966	0.0966	0.0966	0.0975	0.0152	0.0076	0.0076	0.0152								
CLZ	0.2905	0.0966	0.0966	0.0966	0.0966	0.0966	0.0975	0.0152	0.0076	0.0076	0.0152	0.0000							
CSUB	0.2905	0.0966	0.0966	0.0966	0.0966	0.0966	0.0975	0.0152	0.0076	0.0076	0.0152	0.0000	0.0000						
UKN	0.3405	0.3209	0.3209	0.3209	0.3209	0.3209	0.3405	0.3632	0.3487	0.3487	0.3632	0.3602	0.3602	0.3602					
AF126829	0.3047	0.3162	0.3162	0.3162	0.3162	0.3162	0.3346	0.3573	0.3432	0.3432	0.3573	0.3546	0.3546	0.3546	0.1631				
CALB	0.3375	0.3320	0.3320	0.3320	0.3320	0.3320	0.3459	0.3140	0.3012	0.3012	0.3140	0.3120	0.3120	0.3120	0.2864	0.3699			
AF126822	0.3127	0.3346	0.3346	0.3346	0.3346	0.3346	0.3632	0.3032	0.2905	0.2905	0.3032	0.2887	0.2887	0.2887	0.2394	0.3155	0.0470		
GMN	0.4134	0.4384	0.4384	0.4384	0.4384	0.4384	0.4218	0.4305	0.4143	0.4143	0.4305	0.4109	0.4109	0.4109	0.3853	0.3699	0.2888	0.2295	

ence from the cytochrome b marker show that three out of the five farms surveyed have what are mixtures of exotic *C. gariepinus* and *C. anguillaris* (Figure 1), and not a "single species". We argue that since the two species occur sympatrically and cannot be distinguished morphologically, the Dutch domesticated *C. gariepinus* might be a hybrid of the two species. This is possible because of the several sources of the strain including West Africa where they occur sympatrically. Both species have been shown to hybridize in captivity and in the wild (Agnese et al., 1997). Scientists were able to correctly differentiate *C. gariepinus* and *C. anguillaris* only recently (e.g., Agnésé et al., 1997; Teugels, 1982, 1986). A second possibility is a likely contamination of the exotic gene pool with local strains of *C. anguillaris* associated with unwholesome hatchery practices. Possible reasons for contamination include advertent or inadvertent use of species misidentified as *C. gariepinus* by hatchery operators and contam-

ination of facilities with wild fish. Other reasons are economic including (1) it is cheaper to obtain *Clarias* from the wild than maintaining brood stocks; and (2) the cost of exotic *C. gariepinus* fingerlings are about 40% higher than those sold as *C. gariepinus*. Fish seed from contaminated hatchery stocks can experience severe reduction in production indices such as growth, feed conversion efficiency and adaptability. It can also manifest in cannibalism because of likely differential growth rates. This is expected to be more common if species from different populations or two different species are farmed together. Differential growth rates of catfish and resulting cannibalism in aquaculture facilities have discouraged many Nigerian farmers. Contamination can result in the alteration of environment-genetic relationship, and such disruption ultimately lowers fitness and decrease performance (Brannon, 1993).

This study has important implication for *Clarias* aquaculture in Nigeria. And we conclude that further studies which are going on be carried out to determine the genetic status of the exotic *C. gariepinus* by including populations from The Netherlands, Central Africa and Israel as reference populations. Present study has shown that there are two species which constitute the exotic *C. gariepinus* in Nigeria. Aquaculture species must be properly identified and classified to preserve their germplasm and monitor genetic changes.

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